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     FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 19:26:26 ON 13 APR 2005
L1          4122 S "RECOMBINATION SITE" OR "SITE-SPECIFIC RECOMBINATION"
L2         12481 S LOX OR ATT OR FLP
L3        447805 S ANTIBIOTIC OR KANAMYCIN OR AMPICILLIN OR CHLORAMPHEN
L4         36115 S HARTLEY?/AU OR BRASCH?/AU OR BYRD?/AU OR WELCH?/AU OR CHESTNU
L5         60373 S HARTLEY?/AU OR BRASCH?/AU OR BYRD?/AU OR WELCH?/AU OR CHESNUT
L6             0 S L1 AND L2 AND L3 AND L5
L7            25 S L1 AND L5
L8            30 S L2 AND L5
L9           666 S L3 AND L5
L10           93 S RECOMBINATIONAL CLONING
L11            0 S L9 AND L10
L12           20 S L10 AND L5
L13           12 DUP REM L12 (8 DUPLICATES REMOVED)
L14            0 S L13 NOT PY>=1998
L15           33 S L7 OR L8 NOT PY>=1998
L16           16 DUP REM L15 (17 DUPLICATES REMOVED)
L17          653 S "ANTIBIOTIC RESISTANCE GENE"
L18            6 S L17 (P) L2
L19            2 DUP REM L18 (4 DUPLICATES REMOVED)
L20            0 S L17 AND L6
L21            2 S L17 AND L5
L22            2 DUP REM L21 (0 DUPLICATES REMOVED)
L23           30 S L3 AND L17 AND L1
L24            9 S L23 NOT PY>=1998
L25            3 DUP REM L24 (6 DUPLICATES REMOVED)

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L19 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998133161 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9472558
TITLE: A new DNA vehicle for nonviral gene delivery: supercoiled minicircle.
AUTHOR: Darquet A M; Cameron B; Wils P; Scherman D; Crouzet J
CORPORATE SOURCE: UMR 133 CNRS/Rhone-Poulenc Rorer, Vitry sur Seine, France.
SOURCE: Gene therapy, (1997 Dec) 4 (12) 1341-9.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980303

AB Plasmids currently used for nonviral gene transfer have the disadvantage of carrying a bacterial origin of replication and an **antibiotic resistance gene**. There is, therefore, a risk of uncontrolled dissemination of the therapeutic gene and the **antibiotic resistance gene**. Minicircles are new DNA delivery vehicles which do not have such elements and are consequently safer as they exhibit a high level of biological containment. They are obtained in E. coli by **att** site-specific recombination mediated by the phage lambda integrase. The desired eukaryotic expression cassette, bounded by the lambda attP and attB sites was cloned on a recombinant plasmid. The expression cassette was excised in vivo after thermoinduction of the integrase gene leading to the formation of two supercoiled molecules the minicircle and the starting plasmid lacking the expression cassette. In various cell lines, purified minicircles exhibited a two- to 10-fold higher luciferase reporter gene activity than the unrecombined plasmid. This could be due to either the removal of unnecessary plasmid sequences, which could affect gene expression or the smaller size of mini-circle which may confer better extracellular and intracellular bioavailability and result in improved gene delivery properties.

L19 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 84212232 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6233260
TITLE: Method for determining whether a gene of Escherichia coli is essential: application to the polA gene.
AUTHOR: Joyce C M; Grindley N D
CONTRACT NUMBER: GM 28550 (NIGMS)
SOURCE: Journal of bacteriology, (1984 May) 158 (2) 636-43.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198406
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19840628

AB We have developed a general method for determining whether a gene of Escherichia coli is essential for viability. The method requires cloned DNA spanning the gene in question and a reasonably detailed genetic and physical map of the cloned segment. Using this information, one constructs a deletion of the target gene in vitro. For convenience, the deletion can be marked by an **antibiotic resistance gene**. A DNA segment containing the deletion is then cloned onto an **att** delta phage lambda vector. Integration of this phage, by homologous recombination at the target locus, and subsequent excision provide an efficient route for crossing the marked deletion onto the bacterial chromosome. Failure to delete the target gene indicates either that the resulting deletion was not viable or that the desired

recombinational event did not take place. The use of prophage excision to generate the deletion allows one to estimate the fraction of deletion-producing events by analysis of the other product of the excision, the phage produced on induction of the prophage. In this way one can determine whether failure to recover a particular chromosomal deletion was due to its never having been formed, or, once formed, to its failure to survive. Applying this method to the polA gene, we found that polA is required for growth on rich medium but not on minimal medium. We repeated the experiment in the presence of plasmids carrying functional fragments of the polA gene, corresponding to the 5'-3' exonuclease and the polymerase-3'-5' exonuclease portions of DNA polymerase I. Surprisingly, either of these fragments, in the absence of the other, was sufficient to allow growth on rich medium.

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L22 ANSWER 1 OF 2 MEDLINE on STN
ACCESSION NUMBER: 2005151817 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15784541
TITLE: Antibiotic-free plasmid stabilization by operator-repressor titration for vaccine delivery by using live Salmonella enterica Serovar typhimurium.
AUTHOR: Garmory Helen S; Leckenby Matthew W; Griffin Kate F; Elvin Stephen J; Taylor Rosa R; **Hartley M Gill**; Hanak Julian A J; Williamson E Diane; Cranenburgh Rocky M
CORPORATE SOURCE: Defence Science and Technology Laboratory, Porton Down, Salisbury, Wiltshire, United Kingdom.
SOURCE: Infection and immunity, (2005 Apr) 73 (4) 2005-11. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050324
Last Updated on STN: 20050401

AB Live, attenuated bacteria are effective vectors for heterologous antigen delivery. However, loss of heterologous gene-bearing plasmids is problematic, and antibiotics and their resistance genes are not desirable for in vivo DNA vaccine delivery due to biosafety and regulatory concerns. To solve this problem, we engineered the first vaccine delivery strain that has no requirement for antibiotics or other selectable marker genes to maintain the recombinant plasmid. This model strain of Salmonella enterica serovar Typhimurium, SLDAPD, uses operator-repressor titration (ORT) technology, which requires only the short, nonexpressed lacO sequence for selection and maintenance. SLDAPD, recovered from the spleens and Peyer's patches of mice following oral inoculation, was shown to maintain a plasmid that, in contrast, was lost from parental strain SL3261. We also demonstrated successful application of this technology to vaccine development, since SLDAPD carrying a plasmid without an **antibiotic resistance gene** that expressed the Yersinia pestis F1 antigen was as efficacious in protecting vaccinated mice against plague as the parental SL3261 strain carrying an antibiotic-selected version of this plasmid. Protection of mice against plague by immunization with Salmonella expressing F1 has previously required two or more doses; here we demonstrated for the first time protective immunity after a single oral immunization. This technology can easily be used to convert any suitable attenuated strain to an antibiotic-free ORT strain for recombinant protein vaccine delivery in humans.

L22 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:233580 BIOSIS
DOCUMENT NUMBER: PREV200200233580
TITLE: Integron gene sequences within poultry farms and processing plants.
AUTHOR(S): Roe, M. T. [Reprint author]; **Byrd, A.**; Smith, D.; Pillai, S. D. [Reprint author]
CORPORATE SOURCE: Texas A and M University, College Station, TX, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 579-580. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology. ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Apr 2002
Last Updated on STN: 10 Apr 2002

AB The dissemination of multiple antibiotic resistant bacteria is a major issue in the poultry industry. It has been shown that the integron gene sequence plays an important role in the transfer of antibiotic resistance cassettes between pathogens. The objective of this study was to examine

the occurrence of integron gene sequences within poultry production and processing. Microbial community DNA from chicken carcass wash samples at key points within poultry farm and processing plants were PCR analyzed utilizing primers specific for the 5' and 3' conserved segments of the integron gene sequence to determine the presence of integrons in sample DNA. Out of 96 total samples, 51% of the samples were positive for the integron sequence. Seventy one % of on farm samples were integron positive, while, 66%, 46%, and 42% were positive at the post-feather removal, pre-chill immersion, and post-chill immersion location of a processing plant respectively. When 2 independent chiller immersion tanks were sampled at 4 discrete points over a 48-hour period, integron sequences were repeatedly detectable. These results indicate that integron sequences are widely distributed within poultry production and processing. Thus, there are multiple locations at which lateral gene transfer of **antibiotic resistance gene** cassettes can potentially occur.

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L25 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998133161 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9472558
TITLE: A new DNA vehicle for nonviral gene delivery: supercoiled minicircle.
AUTHOR: Darquet A M; Cameron B; Wils P; Scherman D; Crouzet J
CORPORATE SOURCE: UMR 133 CNRS/Rhone-Poulenc Rorer, Vitry sur Seine, France.
SOURCE: Gene therapy, (1997 Dec) 4 (12) 1341-9.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980303

AB Plasmids currently used for nonviral gene transfer have the disadvantage of carrying a bacterial origin of replication and an **antibiotic resistance gene**. There is, therefore, a risk of uncontrolled dissemination of the therapeutic gene and the **antibiotic resistance gene**. Minicircles are new DNA delivery vehicles which do not have such elements and are consequently safer as they exhibit a high level of biological containment. They are obtained in E. coli by att **site-specific recombination** mediated by the phage lambda integrase. The desired eukaryotic expression cassette, bounded by the lambda attP and attB sites was cloned on a recombinant plasmid. The expression cassette was excised in vivo after thermoinduction of the integrase gene leading to the formation of two supercoiled molecules the minicircle and the starting plasmid lacking the expression cassette. In various cell lines, purified minicircles exhibited a two- to 10-fold higher luciferase reporter gene activity than the unrecombined plasmid. This could be due to either the removal of unnecessary plasmid sequences, which could affect gene expression or the smaller size of mini-circle which may confer better extracellular and intracellular bioavailability and result in improved gene delivery properties.

L25 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 97176412 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9023958
TITLE: A recombinase-mediated system for elimination of **antibiotic resistance gene** markers from genetically engineered Bacillus thuringiensis strains.
AUTHOR: Sanchis V; Agaisse H; Chaufaux J; Lereclus D
CORPORATE SOURCE: Unite de Biochimie Microbienne, Institut Pasteur, URA 1300 CNRS, Paris, France.. vsanchis@pasteur.fr
SOURCE: Applied and environmental microbiology, (1997 Feb) 63 (2) 779-84.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970407
Last Updated on STN: 19990129
Entered Medline: 19970321

AB A TnpI-mediated **site-specific recombination** system to construct genetically modified Bacillus thuringiensis strains was developed. Recombinant B. thuringiensis strains from which **antibiotic** resistance genes can be selectively eliminated were obtained in vivo with a new vector based on the specific resolution site of transposon Tn4430. For example, a cryIC gene, whose product is active against Spodoptera littoralis, was introduced into B. thuringiensis Kto harboring a cryIA(c) gene active against Ostrinia nubilalis. The

resulting strain had a broader activity spectrum than that of the parental strain. It contained only B. thuringiensis DNA and was free of **antibiotic** resistance genes. This should facilitate regulatory approval for its development as a commercial biopesticide.

L25 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 95302972 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7783631
TITLE: Mobile gene cassettes and integrons: capture and spread of genes by **site-specific recombination**.
AUTHOR: Hall R M; Collis C M
CORPORATE SOURCE: CSIRO Division of Biomolecular Engineering, Sydney Laboratory, New South Wales, Australia..
SOURCE: Molecular microbiology, (1995 Feb) 15 (4) 593-600. Ref: 32
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950726
Last Updated on STN: 19990129
Entered Medline: 19950720

AB An integron is a genetic unit that includes the determinants of the components of a **site-specific recombination** system capable of capturing and mobilizing genes that are contained in mobile elements called gene cassettes. An integron also provides a promoter for expression of the cassette genes, and integrons thus act both as natural cloning systems and as expression vectors. The essential components of an integron are an int gene encoding a site-specific recombinase belonging to the integrase family, an adjacent site, attI, that is recognized by the integrase and is the receptor site for the cassettes, and a promoter suitably oriented for expression of the cassette-encoded genes. The cassettes are mobile elements that include a gene (most commonly an **antibiotic-resistance gene**) and an integrase-specific **recombination site** that is a member of a family of sites known as 59-base elements. Cassettes can exist either free in a circularized form or integrated at the attI site, and only when integrated is a cassette formally part of an integron. A single **site-specific recombination** event involving the integron-associated attI site and a cassette-associated 59-base element leads to insertion of a free circular cassette into a recipient integron. Multiple cassette insertions can occur, and integrons containing several cassettes have been found in the wild. The integrase also catalyses excisive recombination events that can lead to loss of cassettes from an itegron and generate free circular cassettes. Due to their ability to acquire new genes, integrons have a clear role in the evolution of the genomes of the plasmids and transposons that contain them.

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Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	19033	chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S2	3678	lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S3	118257	promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S4	14112	"antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S5	477	lox SAME promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S6	4939	"antibiotic resistance gene"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:41
S7	2	"5527695".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S8	10	(lox SAME promoter) SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S9	41	lox SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S10	4	"6143557".pn. or "5888732".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S11	13	chloramphenicol SAME lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S12	128	"antibiotic resistance gene" SAME chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42

S13	20	("antibiotic resistance gene" SAME chloramphenicol) AND "site specific recombination"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S14	10	chloramphenicol SAME "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S15	56	chloramphenicol AND "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S16	12133	promoter and "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S17	84016	"immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:42
S18	179	promoter and "antibiotic resistance" and (lox SAME promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:43
S19	19	(promoter and "antibiotic resistance" and (lox SAME promoter)) and "immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/09/17 13:43
S21	21174	chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S22	4137	lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S23	128789	promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S24	15612	"antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S25	577	lox SAME promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31

S26	5370	"antibiotic resistance gene"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S27	2	"5527695".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S28	16	(lox SAME promoter) SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S29	54	lox SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S30	4	"6143557".pn. or "5888732".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S31	18	chloramphenicol SAME lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S32	144	"antibiotic resistance gene" SAME chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S33	24	("antibiotic resistance gene" SAME chloramphenicol) AND "site specific recombination"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S34	15	chloramphenicol SAME "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S35	71	chloramphenicol AND "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S36	13426	promoter and "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S37	87714	"immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31

S38	227	promoter and "antibiotic resistance" and (lox SAME promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31
S39	25	(promoter and "antibiotic resistance" and (lox SAME promoter)) and "immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/04/13 12:31